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Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy:

a 5 year follow-up of 150 patients

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Abstract

One hundred and fifty patients with sustained virologic response (SVR) after treatment of chronic hepatitis C were enrolled in a long-term clinical follow-up study; patients were followed for 5 years for liver-related outcomes and evidence of biochemical or virologic relapse. Patients with stage two or greater fibrosis on pre-treatment biopsy were invited to undergo a long-term follow-up biopsy after their 4th year of follow-up. One hundred twenty-eight patients (85%) were followed through their 4th year and long-term follow-up biopsies were obtained from 60 patients (40%). Forty-nine patients had paired pre-treatment and long-term follow-up biopsies blindly rescored. Forty of these (82%) had a decrease in fibrosis score and forty-five (92%) had a decrease in combined inflammation score. Ten patients (20%) had normal or nearly normal livers on long-term follow-up biopsy. Two patients with pre-treatment cirrhosis developed hepatocellular carcinoma (HCC) and one died. All the other patients with pre-treatment cirrhosis or advanced fibrosis had improved fibrosis scores on long-term follow-up biopsy. No patient had conclusive evidence of virologic relapse. Three patients had persistently elevated alanine aminotransferase (ALT) levels; two of these had new liver disease.

In conclusion, in this cohort of 150 patients with SVR followed for five years the majority of patients had good outcomes. Serum virologic relapse was not seen but two patients with pre-treatment cirrhosis developed HCC and one died. In blind rescoring of forty-nine paired pre-treatment and long-term follow-up biopsies 82% improved fibrosis scores and 92% improved at least one component of inflammation. A minority of patients had normal or nearly normal liver

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Conflicts of interest

Drs. George and Mihindukulasuriya, and Joyce Hoffmann have no conflicts of interest to report. Dr. Bacon has served on advisory boards for Schering Plough and received speaking fees, consulting fees, and research support from Schering Plough, Roche Laboratories, and Gilead Sciences. Dr. Di Bisceglie has served as a consultant for Schering Plough and has received research support from and served on advisory boards for Roche Laboratories. Dr. Brunt has received research support from Roche.

tissue on long-term follow-up biopsy. Patients with cirrhosis pre-treatment are at a low but real risk of HCC after SVR.

Keywords

Hepatitis C; Sustained Virologic Response; fibrosis; inflammation; hepatocellular carcinoma

While the near-term benefit of a sustained virologic response (SVR) after treatment of chronic hepatitis C (HCV) infection is well-established (1;2), knowledge is still incomplete regarding the long-term clinical, virologic, biochemical, and histologic outcomes after SVR (3-8). In particular, the risk of late virologic relapse and late sequelae of HCV infection including hepatocellular carcinoma (HCC) and decompensated liver disease are not known. While previous studies have demonstrated improvement by histologic evaluation >1 year after end of treatment (EOT) (3-5;9-11), the long-term histologic benefit of SVR has not been studied in large patient populations. This outcome is of critical importance, as the ultimate goal of anti-HCV therapy is prevention of cirrhosis and death from liver disease.

Several studies have followed patients for clinical, virologic, and biochemical outcomes for up to ten years after SVR. Comparisons among these studies are limited by differing patient populations, treatment regimens, and HCV RNA detection methodologies. Infrequent late virologic relapse has been reported (3;5;9;11;12) as has persistent post-SVR aminotransferase elevation (3-5;13). One group reported late decompensated liver disease after SVR (14) and two studies reported late death from liver disease (6;14). Finally, Veldt et al followed 142 patients with SVR and advanced fibrosis pre-treatment and found a 2% risk of HCC (6).

The majority of studies which performed histologic evaluation of patients with SVR $\geq$ 1 year after EOT have reported improved scores (3-5;9-11). Comparisons among these studies are limited by the differing lengths of time from EOT the biopsies were obtained and the different scoring methods used. Most groups report more rapid and dramatic improvements in inflammation scores compared with fibrosis scores (3;5;7;10;11;13). Marcellin et al reported no significant change in fibrosis (3), while two other groups noted improvement in fibrosis scores in 25% and 29% of patients, respectively (7;14). The majority of biopsies in these studies were obtained within two years after EOT (3;7;14). In contrast, other groups have reported significant improvements in fibrosis scores in a majority of patients and occasional resolution of fibrosis (4;5;7;9-11;13). Six groups have published a small number of patients with worse liver histology on follow-up biopsy (3-5;7;13;14).

Taken together, these studies indicate that patients with an SVR have a significant chance of histologic improvement in both fibrosis and inflammation scores but may still be at low risk of late clinical sequelae of HCV infection, or virologic or biochemical relapse. However conclusions from these studies are compromised by the often small numbers of patients (3;4;9) or reported brief duration of follow-up (3;7;9). Critically, the long term histologic outcome of people with SVR after HCV therapy has not been studied in a large cohort of patients. To answer this question we enrolled 150 patients in a 5 year clinical follow-up study in which liver biopsies were requested from patients with stage 2 or greater fibrosis pre-treatment after 4 years of follow-up. We determined the long-term clinical, virologic, histologic, and biochemical outcomes of these 150 patients with SVR after treatment of chronic HCV infection.

Methods

Patient enrollment and clinical and biochemical evaluation

Patients with SVR after treatment of chronic HCV (defined as undetectable HCV RNA in serum 6 months after EOT using an approved commercial clinical assay) were invited to enroll. Exclusions were co-infection with either hepatitis B or HIV. Demographic information, treatment history, and HCV genotype data were obtained from medical records. Patients were seen annually for 5 years for a complete history and physical exam by a hepatologist (BRB) and laboratory evaluation including serum HCV RNA, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, alkaline phosphatase, albumin, hemoglobin, white blood cell count, and platelet count. Patients whose pretreatment biopsies were reported as $\geq$ Scheuer stage 2 fibrosis (periportal or focal bridging fibrosis) (15) were invited to undergo a long-term follow-up liver biopsy after their 4th year of follow-up. The study was carried out with the approval of the Saint Louis University Institutional Review Board and written informed consent was obtained from all participants.

Virologic evaluation

Presence or absence of HCV RNA in serum was determined by previously published qualitative RT-PCR methodology with a sensitivity of 100 molecules/mL (16) or 29 IU/mL (17). For this assay, viral RNA was extracted from 140 μ L serum using Qiagen Viral RNA kits in accordance with the manufacturer's instructions. The HCV TMA assay, which has a sensitivity of 5.3 IU/mL (Bayer VERSANT® HCV RNA Qualitative TMA assay package insert) was performed on 0.5mL of the most recent available serum sample according to the manufacturer's instructions (17). Three positive and negative controls were run with each assay. All stages of the assay including sample preparation, target amplification, and amplicon detection were performed in a single tube on a single day by a dedicated operator in a separate room in which no other work was being performed. If the sample was reactive the test was repeated for confirmation and if the results were discrepant the assay was repeated a third time. Only patients who had a serum sample confirmed positive in repeat testing are reported. All stored sera from any patient with a confirmed positive TMA assay were also tested.

Liver biopsy evaluation

Liver biopsies were obtained pre-treatment and during long-term follow-up (defined as at least 4 years after EOT). The initial method of evaluation was for clinical purposes using the Scheuer method (15). At the conclusion of the study, all pretreatment and long-term follow-up biopsies from patients with paired histopathological material available for review were rescored in a blinded fashion using the Ishak system (15) by an experienced hepatopathologist (EMB). Paired biopsies from the two patients who developed HCC were also blindly rescored. The biopsies were randomly ordered and any identifying information on each slide was covered by a randomly generated code number. All the histologic data reported were scored using Ishak and all the conclusions of long term histologic outcomes are drawn based on the Ishak scores of these forty-nine paired biopsies.

Statistics

Non-parametric data such as liver biopsy scoring for fibrosis and inflammation were compared using McNemar Chi-square tests. Means of numeric data were compared using the *t*-test for dependent variables. Analyses were done using STATISTICA software.

Results

Patient demographics, HCV genotype, and treatment history

One hundred fifty patients were enrolled; all had completed HCV therapy at Saint Louis University between June 1997 and April 2002. Their demographic, clinical and treatment characteristics are summarized in Table 1. The mean age at entry was 49 years, 49% of subjects were female, 98% were Caucasian, and the genotype distribution was reflective of the US HCV-infected population. The majority of patients (97%) were treated with combination therapy of interferon alpha 2b (IFN α 2b) injected SQ three times weekly and ribavirin (RBV). Ninety-eight patients (65%) were treated for approximately 1 year and 52 (35%) for approximately 6 months.

Clinical outcomes

All patients had at least one follow-up visit. One hundred twenty-eight patients (85%) were followed through their 4th year and 108 patients (72%) returned 5 or more years after SVR. The median duration of follow-up was 65 months (range: 13-86 months). Two patients died during the study, one of recurrent HCC after orthotopic liver transplant (OLT) and one of a non-liver related cause (esophageal cancer). No patient developed decompensated liver disease but two patients with cirrhosis pre-treatment developed HCC, at 24 months and 33 months after EOT, respectively. Both patients were Caucasian males aged 50. Neither had detectable virologic relapse in serum samples collected up to 78 months after EOT by RT-PCR or HCV TMA methodologies. As sixteen patients had cirrhosis pre-treatment and two developed HCC, this represents a 12.5% risk of HCC and an 6.25% risk of death from HCC after SVR in people with cirrhosis pre-treatment. The overall risk of death was 1.3% and the risk of death from liver-related causes was 0.6%.

Virologic outcomes

147 of 150 patients (98%) had at least one serum HCV RNA test by RT-PCR and TMA after SVR. 137 patients (91%) were tested at least three times and the mean number of samples tested per patient was 5 (range: 1-8). The mean time from EOT to last sample tested was 61 months (range: 12-93 months). No patient had detectable HCV RNA in serum by RT-PCR on any sample. Nine patients (6%) had confirmed detectable HCV RNA by TMA testing of a single serum sample, however all other serum samples from these patients had no detectable HCV RNA by TMA assay (Figure 1). A mean of four samples were tested from each of the nine patients (range 3-6) and collection times ranged from 12 months to 75 months after EOT (mean 60 months). The nine TMA positive patients did not differ from the other 141 patients in demographic, clinical, virologic, or treatment characteristics (Table 2). Four of the nine patients had long-term follow-up biopsies but none had pre-treatment histopathological material available for review, thus none could be included in the blinded rescoring. Review of the clinical pathology reports suggests three of the patients had indications of decreased fibrosis and two had indications of decreased inflammation but this data must be interpreted with caution. Crucially, none of the patients had evidence of histologic deterioration on long-term follow-up biopsy and none has had an adverse liver-related clinical outcome. There was no difference between the mean ALT levels during follow-up of the nine HCV TMA positive patients compared with the other 141 patients, nor were ALT levels increased at the time of collection of the TMA-positive serum sample (Figure 1 and Table 2).

Histologic outcomes

146 of 150 patients had pre-treatment liver biopsies, 116 (77%) were initially reported to have Scheuer stage 2 or greater fibrosis on their clinical pathology reports, and 16 had

cirrhosis (stage 4). Sixty patients (40%) had long-term follow-up biopsies (Figure 2). Forty-nine of these patients, including eight patients with cirrhosis pre-treatment and four with advanced fibrosis, had paired pre-treatment and long-term follow-up biopsy material available for review and re-scoring using Ishak. Pre-treatment biopsies were obtained an average of 6 months prior to treatment (range: 0-36 months) and long-term follow-up biopsies were obtained an average of 62 months from EOT (range: 48-78 months). The results of the rescoring using Ishak of the 49 paired pre-treatment and long-term follow-up biopsies are shown in Figure 3 and 4. By blinded analysis of coded biopsies, two biopsies had scores of 0 for fibrosis and all 3 components of inflammation on long-term follow-up biopsy, thus they recovered essentially normal livers after SVR based on histology (patients 3 and 9, Table 3). An additional eight biopsies had a score of 1 in only one category (fibrosis or necrosis) on long-term follow-up, thus by histologic analysis, they had nearly normal livers (Table 3). Two of these patients had advanced fibrosis (Ishak stage 4) pre-treatment.

In the blinded analysis of 49 paired biopsies using Ishak, 39 of 49 biopsies (80%) had a decrease in fibrosis stage. Sixteen (33%) had a 2 point or greater decrease in stage (Figure 3). Two patients with pre-treatment cirrhosis developed HCC; these were the only patients with increases in fibrosis score on long-term follow-up biopsy (Figure 3A). Ten of twelve patients (83%) with advanced fibrosis or cirrhosis pre-treatment had decreased fibrosis scores on long-term follow-up biopsy and eight (66%) decreased their fibrosis scores by two points or greater. The number of patients with stage 2 fibrosis decreased from forty-nine pre-treatment to twenty-three on long-term follow-up ($p=0.01$). Eight patients had pre-treatment cirrhosis; on follow-up biopsies only two did. Six of 49 patients (12%) had fibrosis scores of 0 on long-term follow-up. Representative pre-treatment and long-term follow-up trichrome-stained liver biopsies are shown in Figure 5A and B.

Forty-six of forty-nine patients (94%) had decreased periportal hepatitis scores between pre-treatment and long-term follow-up biopsy; in twenty-nine patients (59%) the decrease was two points or greater (Figure 4A and B). Thirty-one patients (63%) had grade 0 periportal hepatitis on long-term follow-up biopsy. The number of patients with grade 3 or greater periportal hepatitis decreased from 26 (53%) pre-treatment to 3 (6%) on long-term follow-up biopsies ($p=0.02$). Thirty-four patients (69%) had a decrease in necrosis score between pre-treatment and long-term follow-up biopsy; 9 (18%) had a decreased score of two or more points (Figure 4C and D). Seven patients (14%) had grade 0 necrosis on long-term follow-up biopsy. The number of patients with grade 3 or greater necrosis decreased from 15 (31%) pre-treatment to 1 on long-term follow-up biopsies ($p=0.0001$). Forty patients (82%) had decreased portal inflammation scores between pre-treatment and long-term follow-up biopsy; 18 patients (37%) had a decrease of two points or greater (Figure 4E and F). Twelve patients (24%) had grade 0 portal inflammation on long-term follow-up biopsy. The number of patients with grade 3 or greater portal inflammation decreased from 10 (20%) pre-treatment to 2 (4%) on long-term follow-up biopsies ($p<0.0001$). Three patients had increases in both periportal hepatitis and portal inflammation scores. One of these developed HCC, while no adverse liver-related outcomes have occurred in the other two. The only patient who had increases in all three components of inflammation developed HCC. Representative pre-treatment and long-term follow-up H and E-stained liver biopsies are shown in Figure 5C.

Eleven patients who had pre-treatment and long-term follow-up biopsies did not have pre-treatment biopsy material available for blinded rescoring, thus reports issued at the time of biopsy were reviewed (Figure 2). This group did not differ by age, gender, race, genotype, or treatment history from the 49 patients with evaluable pre-treatment and long-term follow-up histopathologic material. Eight of these patients had indications of improved fibrosis scores and nine had indications of improved inflammation on long-term follow-up biopsy

reports, however in the absence of material for coded, blinded analysis this data must be treated with caution.

Biochemical outcomes

All 150 enrolled patients had at least one biochemical evaluation and 136 (91%) had three or more years of laboratory data collected after SVR. There were no significant differences between the first and last collected samples in the mean ALT (21 U/L), AST (21 U/L), AST/ALT ratio (1.12 and 1.08), total bilirubin (0.6 g/dL), albumin (4.4 g/dL), hemoglobin (14.5 g/dL), white blood cell count (6.6 and 7.1 cells/mm³ × 1000), or platelet count (232 and 235 /mm³ × 1000). There was a significant difference between the mean alkaline phosphatase of the first (74 U/L) and last (67 U/L) collected samples (p<0.0001). When the sixteen patients with pre-treatment cirrhosis were evaluated separately, there was also a significant difference between the mean pre-treatment (82 U/L) and long-term follow-up (73 U/L) alkaline phosphatase levels (p=0.02) but no other significant differences in mean laboratory values (data not shown). Three patients had persistently elevated serum ALT (>49 U/L). Two of these patients also had persistently elevated AST (>49 U/L), AST values were not available from the third patient. One developed HCC, one was diagnosed with autoimmune hepatitis on subsequent biopsy, and the third patient has had no adverse liver-related outcomes. However, this patient was the only patient in the rescored dataset with stage 3 fibrosis pre-treatment who did not have a decreased fibrosis score on long-term follow-up biopsy. None of the three had evidence of steatohepatitis on long-term follow-up biopsy.

Discussion

We followed 150 patients for 5 years after SVR after treatment of chronic hepatitis C infection and determined clinical, virologic, histologic, and biochemical outcomes. The median duration of follow-up was 65 months (range: 13-86 months). This is the largest and longest study of successfully treated chronic hepatitis C to date, and the largest database of long-term follow-up biopsies (3-8;12;18). The population in this study differs from most prior studies in the higher percentage of women enrolled (49%) (12). Otherwise, the patients in this study did not differ from an average cohort of patients with chronic hepatitis C in the United States with the exception of the low numbers of African Americans enrolled.

Overall, clinical outcomes were very favorable, similar to previous studies (3-8;12;19;20). No patient developed decompensated liver disease though two patients developed HCC and received OLTs. None of the patients with advanced fibrosis (Ishak stage 4) pre-treatment progressed to histologic evidence of cirrhosis. In contrast, Pradat et al found that cirrhosis developed in 2 of 87 patients who were followed for at least 5 years after SVR (12). Only one patient in our study died of liver-related causes (recurrent HCC after OLT), similar to previous studies (6;14). However, two of the sixteen patients with pre-treatment cirrhosis developed HCC, a rate of 12.5%. This is a higher rate of HCC than reported in other studies (6;14;19-21) and may be due to either sample size given the low number of patients in our study with cirrhosis pretreatment or to the long period of follow-up. Veldt et al reported 3 of 142 patients (2%) with SVR and Ishak stage 4-6 fibrosis pre-treatment developed HCC during follow-up, however these patients were only followed for a median of 1.1 years (interquartile range: 0.3-2.9 years) (6). Other long-term follow-up studies of patients with SVR have also reported low numbers of patients developing HCC (14;19-21), thus the risk of late development of HCC after SVR in patients with advanced fibrosis or cirrhosis pre-treatment is real, though difficult to quantify.

Neither patient who developed HCC in our study had evidence of virologic relapse in sera by RT-PCR or TMA testing; this strongly indicates that the mechanism(s) which mediated

development of HCC in these patients were not directly related to ongoing HCV replication. While we cannot exclude low-level HCV replication in the liver of these patients after SVR, any substantial replication should have been detectable in serum. Both patients were the only patients in the cohort who had an increase in fibrosis scores between pre-treatment and long-term follow-up biopsy. While both patients already had clear histologic evidence of cirrhosis pre-treatment, the extent of parenchymal remodeling increased between pre-treatment and long-term follow-up biopsy, thus their Ishak fibrosis scores increased from 5 to 6. One of the patients who developed HCC improved his combined Ishak inflammation score from 7 pre-treatment to 3 in the non-tumor liver on long-term follow-up biopsy while the other patient's combined inflammation score deteriorated from 2 pre-treatment to 12 in the non-tumor liver on long-term follow-up biopsy.

Despite extensive testing using two different methodologies, RT-PCR and TMA, no patient had conclusive evidence of serum virologic relapse. None of the 147 patients tested had detectable viral replication in serum by RT-PCR assay. This contrasts with prior studies which found a low rate of late virologic relapse by RT-PCR testing (3;5;9;11;12). Many patients in these earlier studies were treated with interferon monotherapy while all our patients received ribavirin, which may account for the difference. Nine patients had one serum sample with detectable HCV RNA by TMA assay but all other serum samples from these patients tested negative. None of these patients had elevated mean ALTs, nor were their ALTs elevated at the time of collection of the TMA positive serum. These patients were infected pre-treatment with 4 different genotypes or sub-genotypes, thus transient TMA positivity during long-term follow-up did not correlate with pre-treatment infection with a particular genotype. As none of these nine patients had detectable HCV RNA by RT-PCR assay during long-term follow-up, we were unable to perform any post-treatment sequence analysis to determine if the transient TMA positivity represented infection with a new genotype. Four of the nine patients had long-term follow-up biopsies, none of which showed evidence of histologic progression of disease on review of the clinical pathology reports. In this study we did not find any clinical relevance of a single positive TMA assay after SVR.

It is well-known that most patients with an SVR normalize their serum ALT, AST, and total bilirubin shortly after discontinuing treatment unless other liver disease is present (3-5;13). This was confirmed in our study: 91% of our patients had biochemical evaluations three years or more after SVR and only three had persistently elevated ALT values. Of these, one developed HCC, one was diagnosed with autoimmune hepatitis on subsequent biopsy, and the third has had no adverse liver-related outcomes to date.

We obtained 60 long-term follow-up biopsies from the 150 patients. The biopsies were obtained an average of 62 months from EOT (range: 48-78 months), making this the largest dataset of late histologic outcomes evaluated after SVR to date. Forty-nine patients, including eight patients with cirrhosis and four with advanced fibrosis pre-treatment, had paired pre-treatment and long-term follow-up biopsies available for blind rescoring. Liver biopsies are inherently small samples of large organs and estimation of collagen deposition and architectural changes can vary even between experienced pathologists (15). In this study, biopsies were considered acceptable for analysis by the pathologist and potential bias was minimized by randomly sorting the coded slides. Forty of 49 patients (82%) had decreased fibrosis scores and 45 (92%) had decreased inflammation scores on long-term follow-up biopsies. Declines in periportal hepatitis (94%) and portal inflammation (82%) were more frequent than declines in necrosis (69%) scores. In comparison, prior studies reported decreases in fibrosis in paired pre-treatment and post-treatment biopsies in 29% (14), 44% (18), and 59% (13) of patients with SVR. The mean time in these studies between biopsies was 1.6 years (14), 2.5 years (18), and 3.7 years (13), respectively. As we found

82% of patients with paired biopsies obtained a mean of 5.2 years apart had decreased fibrosis, the percentage of patients with SVR who have decreased fibrosis post-treatment may increase over time. These studies also reported 87% (18) and 89% (13) of patients had decreased inflammation on paired biopsies, similar to our study.

Of the 12 patients with advanced fibrosis or cirrhosis pre-treatment in the rescored dataset, ten (83%) had decreased fibrosis scores on long-term follow-up biopsy; two-thirds had a two-point or greater decrease in fibrosis score. Cirrhosis was no longer histologically noted in six of eight patients; the other two patients developed HCC. Two of the forty-nine long-term follow-up biopsies were “normal” based on scores of 0 for fibrosis, periportal hepatitis, necrosis, and portal inflammation. One of these had had stage 3 fibrosis pre-treatment. Eight additional patients had nearly normal liver tissue on long-term follow-up biopsy based on having a score of 0 in 3 of the 4 measured outcomes and a score of 1 in the remaining outcome. Thus recovery of normal or nearly normal liver tissue was seen in 10 of 49 (20%) blindly rescored paired biopsies. Other studies have also reported recovery of normal or near-normal liver tissue after SVR (18).

In summary, we enrolled 150 patients with SVR after therapy for chronic HCV in a long-term clinical follow-up study. The majority of patients did very well but two patients with cirrhosis pre-treatment developed HCC two or more years after SVR and one died from recurrent liver cancer after OLT. This represents a 12.5% risk of HCC and a 6.25% risk of death from liver cancer in patients who had cirrhosis pre-treatment. No other late complications of liver disease were seen. All other patients with cirrhosis or advanced fibrosis pre-treatment had improved fibrosis scores on long-term follow-up. No definite serum virologic relapse was seen despite extensive testing. A persistently elevated ALT after SVR was indicative of new liver disease (HCC and autoimmune hepatitis) in two patients. Long-term follow-up biopsies were obtained from 60 patients a mean of 62 months after end of treatment and all but the two patients who developed HCC had evidence of histologic improvement in inflammation, fibrosis, or both. In conclusion, the clinical, virologic, biochemical, and histologic outcomes of patients followed 5 years after SVR is favorable and recovery of normal or nearly normal liver architecture is possible. However patients with cirrhosis pre-treatment remain at risk for late development of HCC.

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List of Abbreviations

SVR	sustained virological response
HCV	hepatitis C virus
HCC	hepatocellular carcinoma
EOT	end of treatment
ALT	alanine aminotransferase

AST	aspartate aminotransferase
RT-PCR	reverse transcription-polymerase chain reaction
TMA	transcription mediated amplification
IFN-αb	interferon alpha 2b
OLT	orthotopic liver transplantation
RBV	ribavirin

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Figure 1.

147 patients had sera available for testing in the Bayer TMA assay and nine had detectable HCV RNA in one sample. All positive samples were reconfirmed by repeat testing at least once. All stored sera from the nine patients with a positive TMA test were also tested by TMA assay. Positive test results are denoted by a + and negative results by a -. The ALT values (normal range: 5-45 U/L) on the date the sample was collected are shown next to the result (+ or -) of the TMA assay of the serum sample collected on that date.

*stage was not reported on 3 pre-treatment biopsies

Figure 2.

146 patients had pre-treatment liver biopsies. 116 patients had fibrosis scores of 2 or greater pre-treatment on clinical pathology reports using the Scheuer system and were eligible for a long-term follow-up biopsy after their 4th year of follow-up. Sixty patients had long-term follow-up biopsies and 49 had paired pre-treatment and long-term follow-up histopathological material available for pathologist review and blind rescoring using Ishak.

Figure 3.

Forty-nine paired pre-treatment and long-term follow-up biopsies were blindly reviewed and re-scored for fibrosis using the Ishak system (scored as stage 0-6). Paired pre-treatment and long-term follow-up biopsy fibrosis scores of patients are shown connected by a line and the number of patients with each outcome is shown next to the line. *These patients developed HCC. A: Patients with pre-treatment fibrosis scores of 4 or greater. B: Patients with pre-treatment fibrosis scores of 3 or less.

Figure 4.

Forty-nine paired pre-treatment and long-term follow-up biopsies were blindly reviewed and rescored using the Ishak system for periportal hepatitis, necrosis, and portal inflammation (each graded on a scale of 0-4). Paired pre-treatment and long-term follow-up biopsy scores are connected by a line and the number of patients with each outcome is shown next to the line. *Indicates a patient who developed HCC. A and B: Paired pre-treatment and long-term follow-up biopsy periportal hepatitis scores: A) patients with grade 3-4 inflammation pre-treatment, B) patients with grade 0-2 inflammation pre-treatment. C and D: Paired pre-treatment and long-term follow-up biopsy necrosis scores: C) patients with grade 3-4 inflammation pre-treatment, D) patients with grade 0-2 inflammation pre-treatment. E and F:

Paired pre-treatment and long-term follow-up biopsy portal inflammation scores: E) patients with grade 3-4 inflammation pre-treatment, F) patients with grade 0-2 inflammation pre-treatment.

Figure 5.

Representative liver biopsies obtained pre-treatment and during long-term follow-up from three patients. A: Top: pre-treatment biopsy: Trichrome stain with Ishak stage 3 fibrosis (portal-to-portal bridging). Bottom: Long-term follow-up biopsy obtained from the same patient 57 months after EOT: Trichrome stain with Ishak stage 1 fibrosis. B: Top: pre-treatment biopsy: Trichrome stain showing Ishak stage 4 fibrosis (portal-to-portal as well as portal-to-central bridging). Bottom: Long term follow-up biopsy obtained from the same patient 67 months after EOT. Trichrome stain showing Ishak stage 0 fibrosis. C: Top: Pre-treatment biopsy: H and E stain with Ishak combined inflammation score of 6: grade 3 periportal hepatitis, grade 1 necrosis, grade 2 portal inflammation. Bottom: Long term follow-up biopsy obtained from the same patient 49 months after EOT. H and E stain with Ishak combined inflammation score of 1: grade 0 periportal hepatitis, grade 1 necrosis, grade 0 portal inflammation.

Table 1

Subject demographics, HCV genotype, and treatment

Feature	No./mean	Percent/range
Age at EOT	48.7 years (mean)	24-70 years (range)
Gender (female)	74	49%
Ethnicity	148 Caucasian	98.6%
	2 African-American	1.4%
HCV Genotype* 1	75	53%
2	49	35%
3	14	10%
4	3	2%
Treatment		
IFN α 2b+RBV	146	97%
PEG-IFN α 2a + RBV	4	3%
Treatment Duration		
Approx. 1 year	98	65%
Approx. 6 months	52	35%

* 141/150 subjects had genotype information available.

Demographic, clinical, virologic, biochemical, and histologic data from nine patients with transiently detectable HCV RNA by TMA assay

Table 2

Patient	Age at EOT (years)	Gender	Race	Genotype	Treatment	mean ALT (U/L)*	Pre-treatment**		Long term follow-up	
							Grade	Stage	Grade	Stage
1	64	M	C	ND***	IFNa2b+ RBV	19	2	3	0	2
2	44	M	C	1a	IFNa2b+ RBV	17	2	3		ND
3	48	F	C	3a	IFNa2b+ RBV	14	2	2	2	1
4	46	M	C	1a	IFNa2b+ RBV	39	3	2		ND
5	45	M	C	2b	IFNa2b+ RBV	14	2	3		ND
6	51	M	C	1a	IFNa2b+ RBV	45	2	3		ND
7	68	F	C	1b	IFNa2b+ RBV	15	3	4	1	1
8	47	F	AA	1b	IFNa2b+ RBV	11	2	1	2	1
9	46	F	C	2b	IFNa2b+ RBV	20	2	1		ND

* mean ALT of all samples collected during long-term follow-up after SVR

** All pre-treatment and long-term follow-up biopsies were scored according to Scheuer and data was extracted from pathology reports. (15)

*** ND= not done

Paired Pre-treatment (Pre) and long-term follow-up (LTFU) histopathology scores of the 10 rescored patients who had normal or nearly normal liver tissue on long-term follow-up biopsy.

Table 3

Patient #	Fibrosis		Periportal Hepatitis		Necrosis		Portal Inflammation	
	Pre	LTFU	Pre	LTFU	Pre	LTFU	Pre	LTFU
1	4	0	2	0	2	1	3	0
2	4	1	3	0	3	0	2	0
3	3	0	3	0	2	0	2	0
4	3	0	3	0	1	1	2	0
5	3	1	3	0	4	0	2	0
6	3	1	2	0	2	0	1	0
7	2	1	2	0	2	0	1	0
8	2	0	4	0	2	1	2	0
9	2	0	3	0	3	0	2	0
10	2	0	2	0	3	1	2	0